

AMENDMENT TO THE SPECIFICATION

Please amend the specification as shown:

Please delete the paragraph on page 8, lines 1-21, and replace it with the following paragraph:

Figure 4 show a summary of structural elements within regions of pB264 involved in replication and conjugal transfer. (A) Schematic representation of pB264, showing relative positions of restriction sites (as in Figure 3), as well as the positions and relative orientations of nine open reading frames (long arrows) and several repeat elements (small arrowheads). ORFs for which a function has been demonstrated are indicated in darker blue. Numbering of the repeat elements is as defined by Kulakov et al. [16]. Numbers in italics indicate single copies of inverted repeat elements, while the paired elements within the direct repeats are indicated by a single label (DR1 or DR2). Below the diagram of pB264 are schematic representations of the regions involved in replication of pB264 [defined as the intersection of the two smallest autonomously replicating plasmids (plasmids 6 and 8) described in Figure 3] and conjugation of pB264 [defined as the intersection of the two smallest conjugative plasmids (18 and 20) described in Figure 3]. Note that only 9 of the 14 nt comprising one copy of inverted repeat 2 are retained within the replication region (far left). (B) Sequence detail of the region near DR1 (SEQ ID NO: 7). The direct repeat is composed of two 30 nt elements (DR1a and DR1b) indicated in uppercase letters. This region also possesses four smaller (16 nt) direct repeat elements, indicated by arrows above the sequence. (C) Sequence detail in the region of DR2 (SEQ ID NO: 8). The two repeat elements that comprise DR2 (DR2a and DR2b) are indicated in uppercase letters. A sequence element that is conserved among pAL5000-type plasmids is nested within the repeat elements (underlined). Note that the smallest autonomously replicating plasmid bears only DR2a and a single copy of the pAL5000 element and extends only to the position marked “deletion terminus.”

Please delete the paragraph on page 41, line 20, to page 42, line 4, and replace it with the following paragraph:

Construction of pAL311 and pAL312. The plasmid pJP10 was obtained from J. Parker (MIT Dept. of Biology). This plasmid contains the NG2 origin of replication from pEP2, the RP4 *mob* element from pSUP301, a spectinomycin resistance marker derived from the omega interposon, a KanR marker from pUC4K, and *lacI^r* and the *trc* promoter from pTrc99A (Amersham Pharmacia, Piscataway, NJ). pJP10 also possesses a useful polylinker positioned downstream of the *trc* promoter. Deleting the KanR marker and the *lacI^r* gene as a *PvuII* fragment from pJP10 produced pAL307. A 0.7 kb portion of ORF7 was amplified from pB264 by polymerase chain reaction using the primers ORF7 3' (5'-GGAATTCGCTGTCATGAGTGGGCAGGT-3'; SEQ ID NO: 9) and ORF7 5' (5'-CCTGCAGAAATGCCCAGGTTTCGTTCGAGA-3'; SEQ ID NO: 10) and ligated into pCR2.1-TOPO using the TOPO-TA Cloning Kit (Invitrogen, Carlsbad, CA), creating the plasmid pAL308. The ORF7 fragment was then positioned downstream of the *trc* promoter by ligating the *PstI-SpeI* fragment from pAL308 into the *PstI* and *SpeI* sites of pAL307, to make the plasmid pAL311. pAL312 was prepared by ligating an *AatII-SmaI* fragment from pAL298 SXBΔ29Nar (plasmid 8, Figure 3) into the *AatII* and *SpeI* (blunted with the Klenow fragment of DNA polymerase I) sites of pAL311, which reconstitutes ORF7.

Please delete Table 4 on pages 56-57, and replace it with the following Table:

Table 4: Oligonucleotide PCR primers

Name	Sequence (5'-3')	Anneals to (in 5'-3' orientation)
KO1	CAC ACC TAA ACT GAC ATG CT (<u>SEQ ID NO: 11</u>)	Rhodococcus sp. I24 genomic DNA
KO2	GCA ATA TGG TCT TCG CCT (<u>SEQ ID NO: 12</u>)	Rhodococcus sp. I24 genomic DNA
KO3	CGA GAT CGA GAG CAA CAC CA (<u>SEQ ID NO: 13</u>)	pAL302 nt 5066-5085
KO4	CGT TGT AAA ACG ACG GCC A (<u>SEQ ID NO: 14</u>)	pAL302 nt 5552-5534
hpNIMAUP	AAA AAA CAT ATG AGC ACA TCG ACG TCG ACG ACG ACC (<u>SEQ ID NO: 15</u>)	Rhodococcus sp. I24 genomic DNA
hpNIMADO	AAA AGG ATC CTC AGC CGG CGA GGG TGA GCC GGC CG (<u>SEQ ID NO: 16</u>)	Rhodococcus sp. I24 genomic DNA

Please delete Table 5 on pages 66-67, and replace it with the following Table:

Table 5 Primers used for sequencing and PCR

<i>Number</i>	<i>Primer Name</i>	<i>Sequence (5' to 3')^a</i>	<i>Comments</i>
1	pAL298 5'	CGAGATCGAGAGCAA CACCA (SEQ ID NO: 13)	Anneals 5' to gene in pARF50 and pAR51
2	pAL298 3'	GCAAGGCGATTAAGTT GGGT (SEQ ID NO: 17)	Anneals 3' to gene in pAR50 and pAR51
3	<i>nimB</i> forward	CGAAGACCATATTGCT CACCGA (SEQ ID NO: 18)	Anneals 5' to <i>nimB</i> in KY1 genome
4	<i>nimB</i> reverse	GGACTCGAAGATCATC TCGT (SEQ ID NO: 19)	Anneals 3' to <i>nimB</i> in KY1 genome
5	ORF5468 forward	CGTACTGGTTGAACTG GGT (SEQ ID NO: 20)	Anneals to 5' end of ORF5468 in KY1 genome
6	ORF5468 reverse	GGAGAAGATCGTCGAT GTCGT (SEQ ID NO: 21)	Anneals to 3' end of ORF5468 in KY1 genome

^a Primers were ordered from Integrated DNA Technologies (Coralville, IA)